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Screening of Wheat (*Triticum aestivum* L.) genotypes for Heat Tolerance Trait by physio-molecular Markers

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ABSTRACT: The climate change in India lead to the reduction in wheat (*Triticum aestivum* L.) production is often impaired by heat stress. An investigation was carried out to evaluate the yield and yield attributing traits of wheat genotypes under heat stress regimes. An investigation was carried out to evaluate locally cultivated wheat genotypes for heat tolerance based on physio-molecular markers. Heat treatment was given by delayed sowing 30 days from the date of normal sowing. Morphological and biochemical character like chlorophyll content, days to heading, spike length, plant height, 1000 per grain weight and grain yield were recorded for 10 wheat cultivars. Potential of SSR markers for identifying and discriminating 10 wheat genotypes using 10 SSR primers. Amongst these 7 primers amplified distinct band after the PCR operation, WMC153 and GWM140 has found less polymorphic primer rather than WMC198 or WMC266. The SSR primers generated total number of 118 amplicons in 10 genotypes. Amongst these, 68 amplicons were found to be polymorphic with an average polymorphism 56.88 percent.

Keywords: Wheat, heat tolerance, physiological markers, molecular markers.

INTRODUCTION

High temperature (>30C) at the time of grain filling is one of the major constraints in increasing productivity of wheat in tropical countries like India. Hence, wheat genotypes from national and international sources are regularly evaluated for tolerance to high temperature stress. Under such conditions, magnitude of heat stress determines accuracy of evaluation and selection of genotypes. Mean ambient temperatures during the day, month or crop season are often used to quantify the magnitude of heat stress. These temperature parameters, however, fail to account for changes in diurnal amplitude. As a con-sequence, actual duration of high temperature stress to which the crop is exposed to remains elusive (Rane *et al*., 2004).

The higher temperature enhanced plant growth, flowering, and maturation. Thus the number of days to booting, heading, anthesis, and maturity of wheat were significantly decreased that varied among the genotypes. Green leaf area and productive tillers/plant were drastically reduced in time under high temperature. The reduced number of grains/spike and smaller grain size resulted from drastic reduction in growth duration were responsible for the yield loss of wheat at high temperature (Rahman *et al.,* 2009). In India, the main concerns of heat stress are at the reproductive stage due to climate change. Since, in Gangetic plain, winter is short, heat stress induction is early; therefore, high temperature at the time of grain filling stage hampered the yield.

During the flowering and grain filling stage, wheat is generally exposed to short periods of high temperature (33–40°C) (Talukder *et al*., 2014). The total global production of wheat has been reached up to 757.6 million tonnes with an annual consumption of 734 million tonnes (FAO, 2018). In India, the cultivated wheat area is 31.45 mha with production and productivity of 107.59 mt and 34.21 q/ha, respectively (Anonymous 2019).Temperature can modify developmental and growth rates in plants by affects the metabolic pathways at every stage of life of wheat (Limbalkar *et al*., 2018; Ali *et al*., 2018). Grain yield of wheat is the complex trait and influenced by various physiological characters directly or indirectly such as number of productive tillers, spike length, 1000 grain weight and number of spikelets per spike (Li *et al.*, 2020). Assessment of heat tolerance at the molecular level is more meaningful than at phenotypic level as the later involves data on morphological traits which are environmental dependent. Available genetic diversity in wheat offers opportunity for the breeders to develop genotypes with wider adaptability having resistance to biotic and abiotic stresses by selection of recombinants of desired genes. The simple sequence repeats (SSR) markers can help breeders to select genotypes carrying gene(s) of interest (Sadat *et al.,* 2013), therefore, molecular maps based on these markers provide the breeders efficient strategies that may optimize time and resources and facilitate their manipulation in segregating plant breeding populations.

MATERIAL AND METHOD

A. Morphological methods

The sowing was done on 29th November (Normal) in Rabi season, 2020. Heat stress was exposed to genotypes by very late sowing on 29th January, 2021. Biochemical characters like chlorophyll content, the chlorophyll were extracted by the method of Holden (1965), morphological characters like days to heading were calculated by method of Pask, (2012), Determination of the number of grains per spike and thousand grain weight were estimated by the method of Pietragalla and Pask, (2012), Plant height were determined by method of Torres and Pietragall (2012).

B. Molecular characterization

DNA extraction: The molecular genomic DNA of all wheat varieties were isolated by CTAB method (Murray and Thompson, 1980).

SSR analysis: A total of 10 primers were used in SSR analysis. The PCR reaction mixture consisted of 20-50 ng genomic DNA, 1x PCR buffer, 2.0 mmol/L MgCl2, 100 µmol/L of each dNTP, 0.1 µmol/L primer and 3U Taq polymerase in a 25 µL volume. The amplification protocol was 94ºC for 5 min to predenature, followed by 45 cycles of 94ºC for 1 min, 56ºC for SSR analysis for 1 min and 72ºC for 1 min, with a final extension at 72ºC for 10 min. Amplification products were fractionated on 2% agarose gel.

Data analysis: RAPD and SSR data were scored (1) for presence and (0) for absence, each band was regarded as a locus. Two matrices, one for each marker, were generated. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes, was constructed using the unweighted pair group method with arithmetic average (UPGMA) 24 through the software NTSYs-pc version 2.11(Table 1, 2).

Table 1.

No.	Varieties	Total Chl A+B	Total Chl A+B	Diff (normal and heat stress)
1	PBN-1449	19.34	17.38	1.96
2	PBN-1666-1	28.62	24.73	3.89
3	PBN4876-2	15.64	11.99	3.65
4	PBN4357	12.79	9.08	3.71
5	PBN-4881	27.77	26.60	1.17
6	PBN-3958	31.56	29.05	2.51
7	PBW-4905	35.83	33.47	2.36
8	PBN-2189	37.23	36.96	0.27
9	HI-1633	12.85	8.91	3.94
10	AKDW-2997-16	9.38	5.99	3.39

Table 2: The 10 wheat cultivars used in the study.

Sr. No.	Genotypes	Sr. No.	Genotypes
1.	PBN-4449	6	PBN-3958
2.	PBN1666-1	7	PBW-4905
3.	PBN-4876-2	8	PBN-2189
4.	PBN-4357	9	HI-1633
5.	PBN-4881	10	AKDW-2997-16

RESULTS AND DISCUSSION

Analysis of morphological characters for heat tolerance trait:

1. Chlorophyll content: The genotype PBN-2189 recorded maximum chlorophyll content (37.23 mg), whereas minimum in genotype AKDW-2997-16 (9.38mg) in normal wheat condition. Likewise the genotype PBN-2189 recorded maximum in (36.96mg) whereas genotype AKDW-2997-16 recorded minimum in (5.99mg) in heat stress wheat condition. Mohammadi *et al.* (2009) found that chlorophyll content in 18 bread wheat genotypes (V1 to V18) ranged from 21.4 to 20.4 under heat stress

2. Days to heading: Days to heading in normal wheat were highly recorded in PBN-3958 (60days) and AKDW-2997-16 (62 days) while minimum HI-1633 (51.days) and PBN-4881 (57 days). Heat stressed wheat were highly recorded AKDW-2997-16 (52 days) while minimum PBN-3958 (40 days) and PBN-4876-2 (40days). Rahman *et al.* (2009) found that the number of days to heading is significantly reduced under high temperature condition (Table 3).

Table 3.

Sr. No.	Varieties	Days to heading*	Days to heading	Difference
1.	PBN-4449	60	48	12
2.	PBN-1666-1	58.5	42	16.5
3.	PBN-4876-2	59	40	19
4.	PBN-4357	58	49	9
5.	PBN-4881	57	49	8
6.	PBN-3958	60	40	20
7.	PBW-4905	59	48	11
8.	PBN-2189	59	46	13
9.	HI-1633	51	50	1
10.	AKDW-2997-16	62	52	10

3. Spike length (cm): Maximum spike length was recorded from 8.5cm to 8.0cm in PBN-3958, PBW-4905 and AKDW-2997-16 under heat stress condition. Rahman *et al.,* (2009) reported that higher temperature enhanced plant growth and forced the maturity, the different growth and development stages of all the wheat genotypes occurred earlier in High Temperature compared to optimum condition conditions (Table 4).

Table 4.

Sr. No.	Varieties	Spike length*	Spike length	Difference
1.	PBN-4449	7	4.8	2.2
2.	PBN-1666-1	7.8	5.9	1.9
3.	PBN-4876-2	7.6	5.3	2.3
4.	PBN-4357	7.2	4.9	2.3
5.	PBN-4881	8	5.3	2.7
6.	PBN-3958	8.5	7.8	0.7
7.	PBW-4905	8	6.2	1.8
8.	PBN-2189	7.9	6.3	1.6
9.	HI-1633	7.2	5.3	1.9
10.	AKDW-2997-16	7	4.9	2.1

4. Plant height: The highest plant height was recorded in PBN-3958 (62.00cm) and the lowest in HI-1633 (74.25cm) under heat stress condition. The minimum was recorded as AKDW-2997-16 (39.8cm) under heat stress conditions. The mean PH ranged from 83 to 111 cm in South Asia. On an average, PH of trials reduced by 20 cm in ME5 compared to ME1 (Mondal *et al*., 2016) (Table 5).

Table 5.

Sr. No.	Varieties	Plant height*	Plant height	Difference
1.	PBN-4449	81.5	40.5	41
2.	PBN-1666-1	80.33	45	35.33
3.	PBN-4876-2	77.33	55.2	22.13
4.	PBN-4357	77.33	42	35.33
5.	PBN-4881	81.17	44.5	36.67
6	PBN-3958	82.5	62	20.5
7.	PBW-4905	81.5	45.5	36
8.	PBN-2189	77.83	48.2	29.63
9.	HI-1633	74.25	42	32.25
10.	AKDW-2997-16	77.8	39.8	38

5. 1000 per Grain weight (g): High Grain weight (g) under normal condition was highly produced in PBN-4357 (45.85g) and AKDW-2997-16 (85.g) while minimum in PBW-4905 (39.20g) was produced heat stressed condition (Table 6).

6. Grain yield (100 sq Ft): In a normal condition high grain yield recorded in PBN-4881 (367g) and least in PBN-4881(311g) while in heat stressed data showed that high grain yield produced in PBN-1449 (223g) and least grain yield produced in PBW-4905 (178g). Prasad, S. *et al.,* (2018) found that more yield in control condition but highly sensitive to heat stress and showed high percent reduction (Table 7).

Similar findings were reported Jadav *et al.* (2021) by screening 06 wheat varieties (HS 562, HD 2967, HD 3086, HI 1544, MACS 6222, WR 544) for heat tolerance by sowing at two different dates of sowing *viz.* 10 November and 15 December respectively.

Molecular study for heat tolerance trait: Ten wheat genotypes were used to study the polymorphism regarding heat tolerance trait. The SSR primers generated total number of 118 amplicons amongst these, 68 amplicons were found to be polymorphic with an average polymorphism 56.88 percent. The SSR primer WMC154 WMC198 showed 100% polymorphism while GWM-133 and GWM140 primer showed only 33.33 % polymorphism. Highest informative marker with The PIC value of 0.97 was WMC-156 while the least informative marker was found to be GWM-140 with PIC value of 0.15 amplified alleles ranged from 1 to 3 in all the wheat genotypes 7 polymorphic primers out of 10 amplified product. Primer WMC-154 and WMC-198 respectively showed 100 polymorphism in all genotypes. Primer GWM-133 and GWM-140 showed 33.33 percent polymorphism respectively (Table 8).

Table 6.

Sr. No.	Varieties	1000 per Grain weight (g)*	1000 per Grain weight (g)	Difference
1.	PBN-4449	42.6	31.72	10.88
2.	PBN-1666-1	42.1	39.89	2.21
3.	PBN-4876-2	45.05	36.12	8.93
4.	PBN-4357	45.85	32.02	13.83
5.	PBN-4881	39.5	32.88	6.62
6.	PBN-3958	42.1	35.76	6.34
7.	PBW-4905	39.2	33.16	6.04
8.	PBN-2189	42.9	33.1	9.8
9.	HI-1633	40.25	31.05	9.2
10.	AKDW-2997-16	45	30.89	14.11

Table 7.

Sr. No.	Varieties	Grain yield (100 sq Ft)*	Grain yield (100 sq Ft)	Difference
1.	PBN-4449	2401.03	2362.8	38.23
2.	PBN-1666-1	3241.22	3203.97	37.25
3.	PBN-4876-2	3230.8	3197.07	33.73
4.	PBN-4357	3464.3	3437.49	26.81
5.	PBN-4881	3059.84	3025.69	34.15
6.	PBN-3958	2323.9	2292.48	31.42
7.	PBW-4905	3009.81	2967.22	42.59
8.	PBN-2189	3796.48	3757.55	38.93
9.	HI-1633	3671.69	3628.73	42.96
10.	AKDW-2997-16	3992.92	3957.67	35.25

Table 8: List of 10 primers used in studies.

Primer		Sequence
WMC198	F	CACGCTGCCATCACTTTTAC
	R	TTGAAGTGGTCATTGTTGCT
WMC154	F	ATGCTCGTCAGTGTCATGTTTG
	R	AAACGGAACCTACCTCACTCTT
WMC156	F	GCCTCTAGGGAGAAAACTAACA
	R	TCAAGATCATATCCTCCCCAAC
WMC 266	F	ATGTATTTACGAGCATCGACCG
	R	ATGGTTACTCAGCCCACATTCA
GWM44	F	GTTGAGCTTTTCAGTTCGGC
	R	ACTGGCATCCACTGAGCTG
WMC245	F	GCTCAGATCATCCACCAACTTC
	R	AGATGCTCTGGGAGAGTCCTTA
GWM133	F	ATCTAAACAAGACGGCGGTG
	R	ATCTGTGACAACCGGTGAGA
GWM140	F	ATGGAGATATTTGGCCTACAAC
	R	CTTGACTTCAAGGCGTGACA
GWM156	F	CCAACCGTGCTATTAGTCATTC
	R	CAATGCAGGCCCTCCTAAC
GWM268	F	AGGGGATATGTTGTCACTCCA
	R	TTATGTGATTGCGTACGTACCC

On the basis of PCR amplification in DNA the primer WMC-198, WMC-156, GWM-133 and GWM-140 produces unique bands wheat genotypes. Unique and polymorphic banding pattern shows that the primer WMC-156 produce polymorphic amplicons whereas the band in lane 1, lane 2, lane 9, lane 10 are absent while in lane 3,4,5,6,7 respectively present. Primer GWM-140 has given unique band with apx. 320 bp size in lane number four and 300bp size band in lane number 1 and shows polymorphic bands in lane 5, 6, 7. Primer WMC-156 produce unique band at 450 bp size in lane 6 and lane 1 while primer 245 produces polymorphic bands with zero unique bands. Primer GWM-133 produces 300 bp size unique bands in lane 4 and polymorphic bands are showed but primer 154 was not recorded with any unique amplicon. Mahesh *et. al*. (2019) screened 19 wheat (*Triticum aestivum* L.) genotypes for their tolerance of heat stress with 17 wheat microsatellite markers, which were capable of detecting 89 alleles with an average of 4.6 alleles per locus. Polymorphism Information Content value ranged from 0.16 for the primer XGWM 516 to 0.83 for DUPW 117 with an average of 0.60.

Clustering on the basis of wheat genotypes: The relationships among wheat cultivars were estimates by a UPGMA cluster analysis of genetic similarity matrices. The composition of clusters obtained using SSR markers (Fig. 1), has revealed groupings in cases.

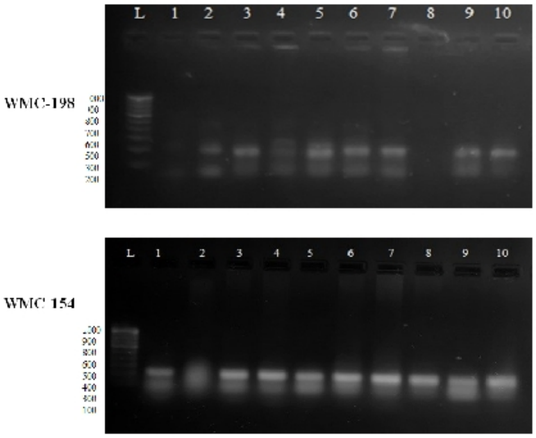

Fig. 1. Polymorphism revealed using SSR primers WMC-198 and WMC-154 to amplify genomic DNA wheat genotypes.

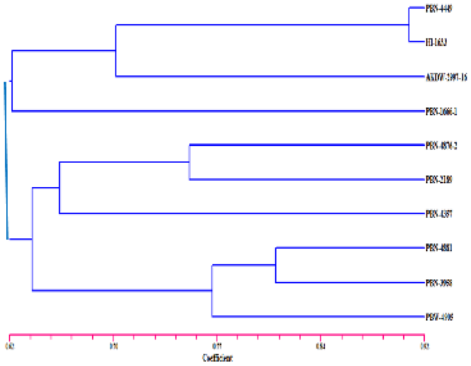

Fig. 2. Dendrogram constructed from similarity coefficients and showing the clustering of wheat genotypes using SSR markers.

Cluster analysis: Cluster I was major cluster with two subcluster in which subcluster I contain two genotypes i.e. PBN-4449, HI-1633 and one outgroup (AKDW-2997-16). Subcluster I with PBN-4449 and HI-1633 sharing 0.9% genetic similarity with each other. Subcluster II contain outgroup with PBN-1666-1 shares 0.58% similarity with AKDW-2997-16.The major cluster-II was further divided in to two sub cluster sharing similarity with each other. The sub cluster IIA contains two genotypes namely PBN-4876-2 and PBN-2189 with one outgroup PBN-4357 shares 0.62% genetic similarity with PBN-2189. The sub cluster II-B contains two genotypes namely PBN- 4881 and PBN-3958 with one ougroup namely PBW-4905 shares 0.73% genetic similarity with PBN-4881. Hassan, M.I. (2016) did cluster analysis of similarities using Jaccard's coefficients based on SSR markers data classified the ten bread wheat genotypes evenly into two groups.

CONCLUSION

The varieties experimented under normal (29th November) and delayed sown conditions to induce heat stress (29th December) revealed that all the varieties sown under delayed condition showed significant decline in biochemical characters like chlorophyll content and morphological characters like days to heading, Spike length (cm), Plant height, 1000 per Grain weight (g) and Grain yield (100 sq Ft) this may be due to short duration of growth under heat stress condition. Normal sown crop produce superior grain yield along with all good quality parameters. Variety AKDW-2997-16 followed by PBN-2189 produced higher grain yield under both normal and heat stress sown condition.

FUTURE SCOPE

This study will help the researcher to identify heat tolerant lines and fasten screening of genotypes for heat tolerance trait by using different physiological and molecular markers.

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Conflict of Interest. None.

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